Observations of length changes in preserved freshwater young fish.

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by

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ABSTRACT

LESLIE, J.K. 1983. "Observations of length changes in preserved freshwater young fish". Can. Tech. Rep. Fish. Aquat. Sci., (1236).

Several body characters of a number of species and sizes of freshwater young fish were measured for immediate, short, and long term effects of preservation. There was both lengthening and shrinkage of body parts. Some small, five mm, fish did not immediately change from their live total length, whereas others shrank two percent during the first two days. Larger, 22 mm, larvae shrank a maximum of five percent in specific body parts during four to six weeks, after which stabilization occurred. There was only slight evidence of continuing length changes after one year; the amount of change varied according to the type of preservative. Davidson's B maintained the morphological integrity of larval fish better than did four percent formalin, ten percent Borealene, and 60 percent ethanol (which was least effective). Most lengths remained static but snout to vent length shrank one to three percent in white sucker larvae in all types of preservative during the short-term period. Long-term preservation in Davidson's B of a number of species of various size classes did not affect length changes of practical consequence.

RÉSUMÉ

LESLIE, J.K. 1983. "Observations sur la variation de longueur de jeunes poissons d'eau douce conservés" Can. Tech. Rep. Fish. Aquat. Sci. (1236).

On a mesuré plusieurs caractéristiques physiques de plusieurs espéces et tailles de jeunes poissons d'eau douce pour déterminer les effets immédiats, à court et à long terme de la préservation. On a constaté que certaines parties du corps allongent et que d'autres rapetissent. Certains poissons de petite taille (5 mm) n'ont pas immédiatement changé par rapport à la longueur totale qu'ils avaient de leur vivant, mais d'autres ont rapetissé de 2% au cours des deux premiers jours. Chez des larves plus grandes (22 mm), certaines parties du corps ont rapetissé de 5% au maximum durant une période de 4 à 6 semaines, après quoi il y a eu stabilisation. Après un an, il y avait très peu d'indices que la longueur continuait de varier; l'importance de la variation dépendait du type de produit de conservation utilisé. Le produit Davidson B assure une meilleure intégrité morphologique des poissons larvaires que le formol à 4%, le Borealine à 10% et l'éthanol à 60% (qui est le moins efficace). La plupart des longueurs sont demeurées stables, mais la longueur museau-orifice anal

a rapetissé de 1 à 3% chez les larves de meunier noir dans tous les types de produits de conservation durant la période de court terme. La conservation à long terme dans le produit Davidson B de plusieurs espèces et tailles n'a pas donné lieu à des variations de longueurs ayant des conséquences pratiques.

INTRODUCTION

Length data of young fish are typically obtained from preserved, rather than live specimens, since immediate examination of captured fish is not always practical and must await the completion, sometimes months later, of field sampling. During the interim, morphological changes in preserved young fish may have occurred. Such changes may interfere with identification and accurate taxonomic description, and could also be inadvertently ascribed to environmental factors or genetic influences. There is a scarcity of published data on preservation effects on freshwater young fish; investigations of this nature have been mostly concerned with marine species (Blaxter 1971; Theilacker 1980; Hay 1982; Fowler and Smith 1983).

This study was intended to document length changes in several species and sizes of Great Lakes young fish that were stored in various preservatives. Some variables on which preservation and storage had limited affect were sought for use in growth estimates. Although immediate effects were examined, changes in length were mainly followed over periods of from two months to more than six years.

MATERIALS AND METHODS

Larval and juvenile fish were selected both from fresh samples and from an extensive collection of preserved specimens for which there were historical data on linear measurements. Fish from prior studies had been fixed on site in formaldahyde that had been neutralized with marble chips and diluted with habitat water to approximately five percent. Within one month of capture, fish were transferred to Davidson's B (formalin, glycerol, 95 percent ethanol, and water in proportions 1:1:5:3, respectively), which has been most commonly used in this laboratory for the past ten years. As the type of preservative is not only chosen for its ability to conserve the morphological integrity of specimens, but also for unrelated factors, e.g., toxicity, cost, perishability, and availability, three other preservatives (60 percent ethanol, four percent neutral formalin, and ten percent Borealene) were tried for comparative purposes for periods of up to about one year.

Young fish did not exceed four percent of the total volume of the preservative, and samples have been dark-stored for up to seven years in tightly sealed glass scintillation vials at room temperature (25°C). All measurements were taken by the author, using a Wild M5 dissecting microscope with an ocular micrometer. Except where specified, lengths are to the nearest 0.1 mm.

Linear measurements of total and standard length (TL, SL), snout to vent (SV) and head (H), were made according to Lippson and Moran (1974). The distance between the orbital rims, or the orbit (O), least depth in the region of the caudal peduncle, greatest body depth (GD), and yolk sac length (YS), were also measured in some fish.

IMMEDIATE EFFECTS

In order to determine possible "immediate" length changes, i.e., during the first few weeks, measurements were made of several fish species alive, then 7, 8, 14, 21, 30 and 45 days after fixation. Twenty laboratory-reared johnny darter, Etheostoma nigrum, and a composite field sample (n = 21) were measured, as were eight larval white sucker, Catostomus commersoni, and 17 blacknose dace, Rhinichthys atratulus. Fish were individually anaesthetized with 50 percent carbonated water, measured, then fixed and preserved in Davidson's B at 25°C.

SHORT-TERM EFFECTS

Length measurements were first made when specimens had been preserved for one or two months, then periodically for the next 16 to

17 months. This procedure covered a time span typical of routine laboratory processing of field samples. For purposes of comparison, the mean lengths of the initial measurements were taken as the "original" lengths.

Two groups of fish were examined. One group of 117 white sucker larvae, which were caught in July, 1980, were equally divided, fixed, and stored in the four types of preservative. Individuals were first measured in September 1980, then during the following 2, 6, 9, 16, 22, 42, and 63 weeks. A second group of 22 juvenile grass pickerel, Esox americanus vermiculatus, was caught in July, 1980, and fixed and preserved in 10 percent Borealene. Linear measurements of each fish were taken during the following 6, 8, 10, 12, 16, 23, 28, 66, 75, and 101 weeks.

LONG-TERM EFFECTS

One hundred larval rainbow smelt, Osmerus mordax, from an entrainment study (Leslie et al., 1979) were measured on two occasions 28 months apart. Representatives of relatively short, medium, and long intestine-species were selected from another group of entrained larvae (Kelso and Leslie 1979). These fish were measured initially in 1975, then re-measured in 1980.

Length measurements in 1974 and 1980 of 50 white sucker and 30 spottail shiner, Notropis hudsonius, from Nipigon Bay (Leslie and Kelso 1977) were compared. A Student's 't'-test ($P = 0.01$) of unpaired samples was applied to the data.

RESULTS

Routine measurement precision was estimated from ten successive measurements of larval fish body characters in 26 size classes, ranging from 1 to 54 mm. The coefficient of variation averaged 0.5 percent throughout the range, and did not exceed one percent in any size class.

IMMEDIATE EFFECTS

In general, fixation and preservation did not induce immediate artifical length changes in several body characteristics of larval fish. Johnny darter larvae (6.1 mm TL) as a group did not change significantly (paired 't'-test; $P < 0.01$) in total or standard length, snout to vent, head, or yolk sac length during 45 days in Davidson's B. However, individual larvae both lengthened and shrank throughout the measurement sequence. The greatest shrinkage

(6.3 percent) and lengthening (3.2 percent) of any individual occurred within two days of fixation.

Fish of the composite sample ($\bar{x}$ = 5.5 mm TL) shrank two percent from live total length during the first two days, lengthened slightly during the following three weeks, then stabilized. This is shown in Figure 1, together with changes (snout to vent and body depth) in white sucker larvae (27.2 mm TL), and blacknose dace (20.9 mm TL) during 45 days. Lengths appeared to stabilize after three or four weeks, with approximately half the total shrinkage occurring during the first two days after fixation. Shrinkage of white sucker in standard length, snout to vent and greatest depth was four to five percent, and significant ('t'-test; $P < 0.01$), as were total and standard length, and snout to vent in blacknose dace, in which negative changes were two to three percent.

SHORT-TERM MEASUREMENTS

The manner in which five body characters of larval white sucker changed in length during several measurement intervals is shown in Figure 2. Larvae preserved in Davidson's B increased slightly in total length and head, but initial dimensions were virtually unchanged during 63 weeks. Total and standard lengths decreased one to two

percent in formalin and in 60 percent ethanol. Snout to vent length shrank significantly (ANOVA F-test; $P < 0.01$), but final measurements showed changes of only -0.8 to -2.6 percent in all preservatives. Of the four preservatives tested, 60 percent ethanol generally effected the greatest negative change. Although shrinkage of the orbit was relatively large, the limits of measurement precision to some extent masked real changes in this small variable. The morphology of all specimens appeared unaffected by preservatives.

There was limited length change in Borealene-preserved grass pickerel ($n = 22$) during each of ten measurement intervals, which covered 101 weeks. Variability in length changes with time was more exaggerated in individual fish than were group changes. Initial total length (52.0 ± 2.7 mm) increased one mm during the first five weeks, decreased an equal amount between weeks 28 and 66, and thereafter slowly lengthened to 101.9 percent of the initial value. Compared with initial measurements, final lengths of head and orbit were 97 percent and least depth was 90 percent. Maximum increase in total length of any individual fish was ten percent, whereas decreases amounted to 17 percent and 8 percent in least depth and orbit, respectively. Two-way analysis of variance of measurements of total length, head, orbit, and least depth between ten dates indicated respective F-ratios of 26.3, 13.6, 20.0, and 21.0, which were significant ($P < 0.01$; $df = 9, 189$).

LONG-TERM MEASUREMENTS

One hundred larval rainbow smelt did not change in total length whilst stored in Davidson's B from June, 1978 to September, 1980. Mean length (±1 SD) in 1978 was 5.7 ± 0.5 mm, and 5.6 ± 0.6 mm in 1980. Similarly, a wide size range of young fish from Lake Huron showed minor or negligible change in total and standard lengths (Table 1) during 65 months.

Larval fish of the same age but of different species and size were affected differently during more than six years of preservation in Davidson's B. Nipigon Bay spottail shiner (Notropis hudsonius) shrank four to six percent in some body parts, whereas white sucker remained essentially the same (Table 2). Changes were not significant (unpaired 't'-test; $P < 0.01$), and all specimens were intact and apparently unaffected from long term preservation.

DISCUSSION

Length changes in young fish due to fixation and preservation are almost universally reported as shrinkage. However, negative change does not necesssarily occur, as Billy (1982) showed in standard length measurements of Sarotherdon mossambicus, fixed in ten

percent formalin and transferred to 37.5 percent isopropyl alcohol. Similarly, the present study noted a number of instances in which there is no change in measurements of body parts of various sizes of several species of young fish during immediate, short, and long-term periods of preservation. Nevertheless, in most cases, shrinkage was the rule.

Although different species were involved in tests of immediate effects and are thus not directly comparable, it was notable that small larval fish shrank proportionally less than large ones. This differs with the findings of Schnack and Rosenthal (1978) and Hay (1982) of shrinkage in Pacific herring, and by Fowler and Smith (1983) of silver hake, in which shrinkages were inversely related to size. During long-term preservation in Davidson's B, white sucker shrank less than their smaller counterpart, spottail shiner. At present there is insufficient evidence to conclude that shrinkage is a function of species or size, however.

The extent of length change largely depends on techniques employed in capture and fixation (Parker 1963; Theilacker 1980), and on many biological considerations, e.g., species, size, age, body form, and condition factor. Operator bias, or experimental error, is probably as significant as most factors in determining the extent of linear changes, especially those involving small parameters.

The orbit and head length appear to be stable and reliable variables but apparent changes in their lengths can be affected considerably by measurement errors, and for this reason, may be unsuitable as growth rate indicators. Snout to vent length, an important identification tool and morphometric variable used directly or in ratio with other characters, appeared more susceptible to shrinkage than were other body parts. On the other hand, total and standard length are used appropriately to estimate growth rates of young fish, since preservation effects are relatively small.

Although length changes accompany weight changes, these relationships are allometric throughout larval fish growth (Kramer and Smith 1960; Kudrinskaya 1973; Phillips and Barraclough 1977). Thus, it remains to be seen if live biomass estimates can be obtained from length data of preserved samples. However, for purposes of identification, descriptive taxonomy, and illustration, lengths of specimens preserved in Davidson's B closely approximate live or original values. Although sometimes statistically significant, changes were invariably of limited practical consequence. It is notable that no changes in gross morphology occurred in specimens. Such changes could be mistakenly attributed to factors unrelated to preservation.

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Figure 2 Short-term length changes in body components of white sucker larvae in various preservatives, expressed as % of initial lengths.

Table 1. Mean lengths (mm ± 1 SD) in 1975 and 1980 of six species of entrained Lake Huron young fish, stored in Davidson's B for 65 months. 1980 values in parentheses

Species	n	Length			
		Total		Standard	
Osmerus mordax	32	5.3±0.4	(5.2±0.4)	5.1±0.4	(5.1±0.3)
Notropis atherinoides	21	5.7±0.3	(5.7±0.2)	5.5±0.3	(5.5±0.2)
Eucalia inconstans	17	7.2±1.1	(7.1±1.2)	6.9±1.1	(6.9±1.1)
Cottus cognatus	19	8.9±0.6	(8.7±0.6)*	7.5±0.5	(7.4±0.5)
Myoxocephalus thompsoni	29	11.3±1.0	(11.1±0.9)*	10.6±0.9	(10.5±0.9)
Alosa psuedoharengus	13	20.1±4.1	(19.9±4.2)	17.6±3.1	(17.5±3.2)
Osmerus mordax	35	34.4±7.5	(34.6±7.8)	29.9±6.4	(29.9±6.4)

* Changes significant ($P < 0.01$).

Table 2. Mean lengths (mm ± 1 SD) of 1974 Nipigon Bay larvae stored in Davidson's B for 75 months. 1980 values in parentheses

Species	n	Morphological Character				
		TL	SL	SV	H	O
Notropis hudsonius	30	9.0±1.9 (8.5±1.5)	8.1±1.5 (7.8±1.2)	5.5±1.0 (5.2±0.8)	1.9±0.5 (1.8±0.4)	0.8±0.2 (0.8±0.1
Catostomus commersoni	50	15.3±1.4 (15.4±1.7)	14.1±1.2 (14.2±1.4)	11.3±1.1 (11.1±1.3)	3.1±0.5 (3.0±0.4)	1.2±0.1 (1.2±0.1)

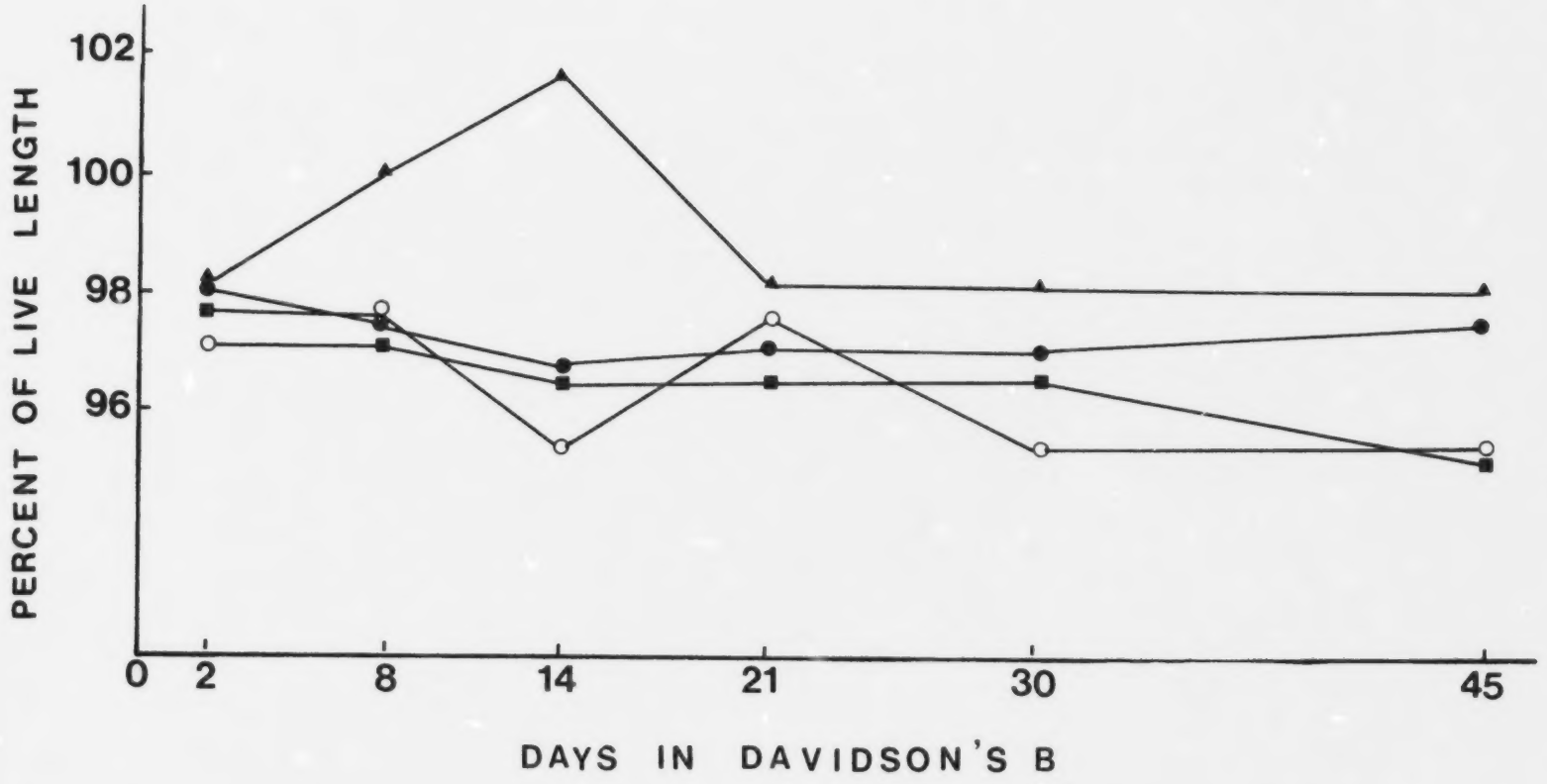

Fig. 1 Immediate changes (% of live value) in white sucker (n = 8) snout to vent length (■), and greatest depth (o); blacknose dace (n = 17) total length (•), and a composite sample (n = 21) total length (▲), all preserved in Davidson's B.

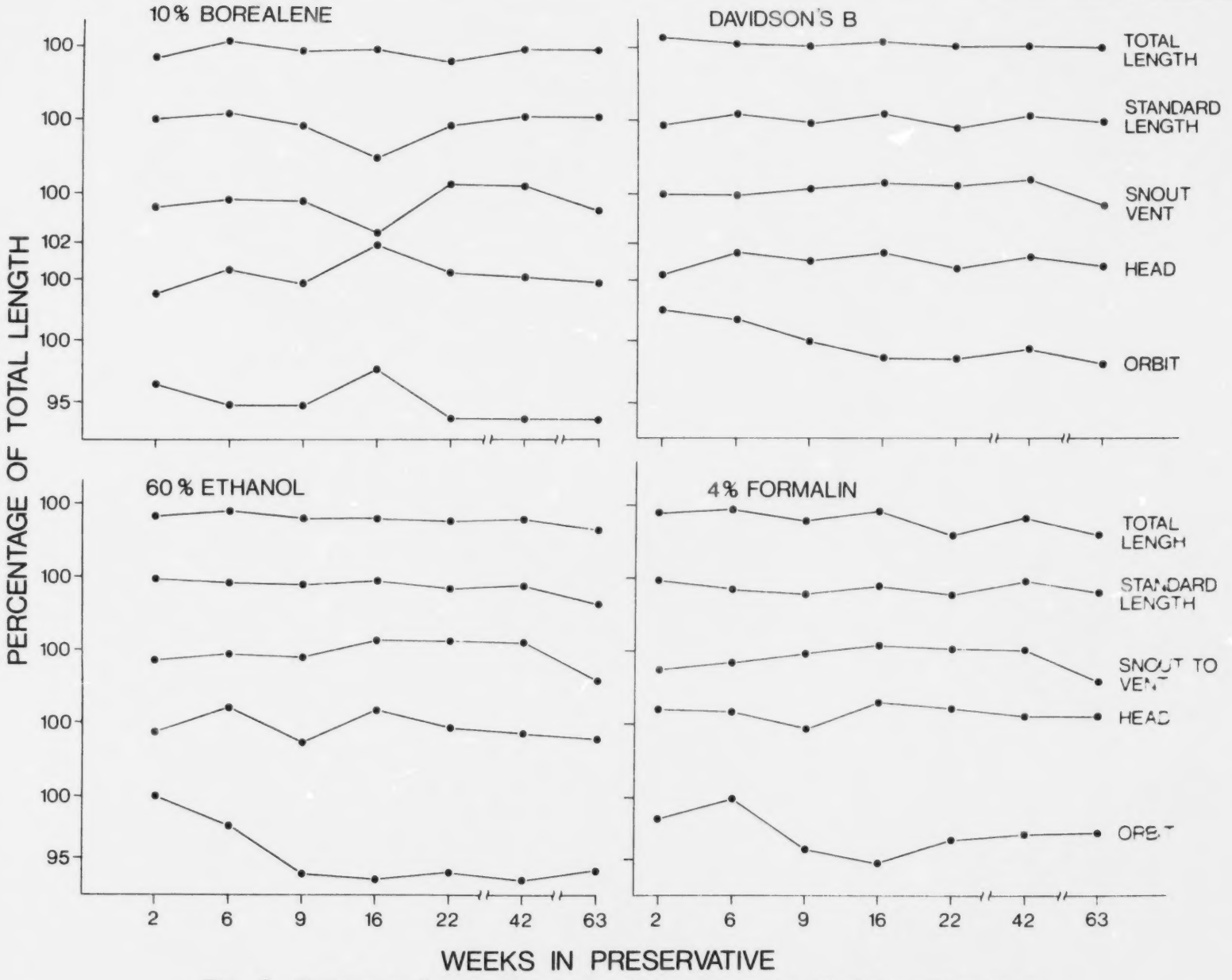

Fig. 2 Short-term length changes in body components of white sucker larvae in various preservatives, expressed as % of initial lengths.